124 Eradication of *Pseudomonas aeruginosa* in children with cystic fibrosis: an Australian experience

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Introduction: Early eradication therapy for *Pseudomonas aeruginosa* (*Pa*) reduces morbidity and mortality in cystic fibrosis (CF) lung disease. Standard *Pa* eradication at Royal Children's Hospital (RCH) is 3 months inhaled tobramycin and oral ciprofloxacin in months 1 and 3. A preceding two weeks of IV antibiotics are given to children <6 years of age or those clinically unwell.
Objective: The aim of this study was to evaluate success of *Pa* eradication using two definitions.
Methods: A retrospective review of eradication success in children acquiring *Pa* between August 2004 and August 2011 was conducted. *Pa* eradication at RCH was defined by

1. no *Pa* on three specimens (sputum or oropharyngeal) obtained at least one month apart after end of treatment; or
2. no *Pa* on BAL and another specimen (BAL, sputum or oropharyngeal) obtained at least one month apart after end of treatment.

Absence of *Pa* in a single specimen obtained ⩾1 month after end of treatment period was also examined for comparison ("single specimen definition").
Results: 101 episodes of *Pa* eradication were reviewed in 57 CF patients (61.4% male). Median age at start of eradication was 7.9 years (range 0.3–17.6 years).

Table 1. Success of Pa eradication at RCH

		Successful eradication		
	No. of patients	RCH definition, n (%)	Single specimen definition, n (%)	Two-tailed p value (Fisher's exact test)
1st eradication	57	41 (71.9)	47 (82.5)	0.26
2nd eradication	28	17 (60.7)	19 (67.9)	0.78
3rd eradication	16	8 (50.0)	12 (75.0)	0.27

Conclusion: We report rates of *Pa* clearance lower than those observed in previous studies. However, defining "eradication" by a single culture result may overestimate treatment success. Our study highlights the need to develop and adopt a standardised and stringent definition for *Pa* eradication.

125 Presence of *Pandoraea* and *Herbaspirillum* species within the Irish CF population

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Objective: To provide molecular characterization of *Pandoraea* and *Herbaspirillum* strains isolated from Irish CF patients.
Materials and Methods: Molecular analysis using the 16S rRNA gene, gyrB gene and rpoB gene in addition to MALDI-TOF MS was used for species characterisation. Clonality was assessed using PFGE and BOX-PCR and the presence of class 1 integrons were also investigated.
Results: Over a study period of 6 years 11 *Pandoraea* positive patients and 12 *Herbaspirillum* positive patients were identified. 16S rRNA gene sequencing confirmed *Pandoraea* species to be *P. pnomenusa* (5 patients), *P. pulmonicola* (3 patients) and 3 patients isolating an as yet unclassified *Pandoraea* sp. Clonal analysis revealed the capacity of *P. pnomenusa* and *P. pulmonicola* to be transmissible from patient to patient. Class 1 integrons containing antibiotic resistance genes were also found which may have contributed to pan-resistance in strains.
Herbaspirillum species were confirmed as *H. huttensie* subsp. *putei* by DNA sequencing and were shown to be part of an incident of transmission among CF and non CF patients.
Conclusion: These reported cases of infection indicate species of *Pandoraea* and *Herbaspirillum* are present in the Irish CF population. While *Pandoraea* species have been shown to possess significant capacity to elicit pro inflammatory response in CF lung cells *Herbaspirillum* species while potentially transmissible have yet to be evaluated for pathogenic impact.

126 Typing of *Prevotella* spp. from CF and patients with other diseases

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Objectives: Anaerobic bacteria from a range of genera have been detected in large numbers in sputum from CF patients with *Prevotella* spp. frequently isolated. *Prevotella* are well known oral pathogens and considered significant in other polymicrobial lung infections. This study aimed to determine whether any species of *Prevotella* are particularly associated with colonisation of the respiratory tract.
Methods: *Prevotella* isolates from CF sputum (n = 52), non-CF clinical isolates from a range of body sites (n = 57; of which n = 7 respiratory and n = 50 non-respiratory) and type strains (n = 5) were cultured under strict anaerobic conditions. Isolates were typed by 16s rDNA sequencing, Pulse Field Gel Electrophoresis (PFGE) and Random Amplification of Polymorphic DNA (RAPD).
Results: 16s rDNA sequencing data grouped isolates (n = 101) into clusters (n = 17) dependent upon species. In contrast, dendrograms produced by analysis of both PFGE (n = 95) and RAPD (n = 103) grouped isolates into clusters (n = 11 and 12, respectively) which contained different *Prevotella* species. Also, the same species appeared in more than one cluster. There was no trend as to the clustering of isolates from either condition (CF vs non-CF) or source (respiratory vs non-respiratory).
Conclusion: The methods used in this study have shown that no particular *Prevotella* species were particularly associated with colonisation of the respiratory tract in either CF or non-CF patients. Both PFGE and RAPD indicated a significant degree of homology between *Prevotella* isolates, which were identified as distinct species by 16s rDNA sequencing. This may suggest limitations to 16s rDNA sequencing for definitive identification and speciation of *Prevotella.*

127 The first report of a cluster of *Bordetella petrii* in an Irish cystic fibrosis population

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Aim: To determine the prevalence and clinical significance of *B. petrii* in cystic fibrosis (CF) patients.
Method: Four patients in the Midwest (MW) of Ireland yielded *Bordetella* species from clinical specimens, these isolates were sent to a reference lab for speciation. Identification was determined by 16s RNA sequencing of a 1407 bp region and MALDI-TOF analysis. Strain relatedness was determined by BOX AIR PCR and Pulsed Field Gel Electrophoresis (PFGE). A follow-up prevalence study was performed anonymously on archived DNA from CF sputae in the Reference Lab contributed by several other CF centres in Ireland using 16s RNA sequencing of 105 CF sputum samples. A retrospective epidemiological investigation of the MW cluster was also undertaken.
Results: Six of 105 were positive for *B. petrii* in the prevalence study which had not been detected by standard culture techniques. All MW isolates were indistinguishable by the above methods. The MW patients were clustered geographically in the same county of Ireland and were all chronically colonised with *Pseudomonas aeruginosa.* The first report of *B. petrii* isolation in the group was in 2009, with all other isolates occurring in 2010/11. All patients had differing baselines of physical condition and were therefore compared through various methods such as FEV_1 trends, Quality of Life Scores, etc. The clinical significance of *B. petrii* was assessed by detailing symptoms at the time of positive sputum result, the presence/absence of co-pathogens on sputum culture and antimicrobial sensitivity of *B. petrii* to the antibiotic regime used.
Conclusion: These findings suggest that this bacterium may be underreported in the CF population.